# Smith-Lemli-Opitz Syndrome; The Bristol Experience

**Mary Gable[a], Julie Honeychurch[a], Mark Greenslade[a], Ann Brown[b], Hilary Sawyer[a] and Maggie Williams[a].**

[a]Bristol Genetics Laboratory, North Bristol NHS Trust, Southmead Hospital, Bristol, BS10 5NB; [b] Biochemical Genetics Unit, North Bristol NHS Trust, Southmead Hospital, Bristol, BS10 5NB

**E-mail:** mary.gable@nbt.nhs.uk **or** julie.honeychurch@nbt.nhs.uk

## Introduction to Smith-Lemli-Opitz Syndrome (SLOS)

•Autosomal recessive, metabolic congenital multiple malformation disorder.
•Phenotype is heterogeneous ranging from intrauterine lethality through to mild mental impairment. Common features include: microcephaly, cleft palate, limb anomalies such as polydactyly, hypospadias or ambiguous genitalia in males and cardiac anomalies.
•Incidence in the UK is between 1/15000 – 1/60000 [2,3,4].
•The carrier frequency could be as high 2% in Caucasian populations [1].
•The gene involved is *DHCR7* (OMIM 602858), which has 9 exons of which exons 3-9 are coding.
•The intron 8 variant c.964-1G>C accounts for approximately 47% of *DHCR7* mutations in the UK [2].
•It encodes the enzyme 7-dehydrocholesterol reductase (DHCR7) which is an integral membrane protein.
•DHCR7 is the final enzyme in the sterol synthetic pathway that converts 7-dehydrocholesterol (DHC7) to cholesterol.

## A Collaborative Service

•Direct analysis of DHC7 levels in plasma and tissue has been available at the Bristol Biochemical Genetics Unit since 1995. Analysis can detect raised levels in amniotic fluid and chorionic villus samples, providing rapid prenatal diagnosis. Analysis can also identify SLOS carriers. Bristol reference ranges: Raised DHC7 levels are > 20µmol/L, atypical levels are >2.5-20 µmol/L.
•In 2009 *DHCR7* gene testing was introduced by this laboratory to provide a comprehensive collaborative SLOS service.
•Gene testing is undertaken following referral from either Clinical Genetics or a Metabolic Specialist. The main testing criterion is raised DHC7 levels.
•Information on the BGL SLOS service and the UKGTN proforma detailing the minimum criteria for testing is available through the BGL website:
•http://www.nbt.nhs.uk/for_gps/services__referral/b/bristol_genetics_laboratory/molecular_genetics.aspx.

## *DHCR7* Mutations Detected at BGL

*DHCR7:* spans 14kb of DNA and is located at 11q12-13. This gene encodes the 7DHC enzyme which is a 475 amino acid transmembrane protein located to the endoplasmic reticulum membrane and nuclear outer membrane. A number of models of the transmembrane topology of this enzyme have been proposed based on the protein sequence, including a 6 and 9 transmembrane domain model [5]. Mutations in the 4th cytoplasmic loop and null mutations are the most severe whereas C terminus and transmembrane mutations are thought to be milder [5].

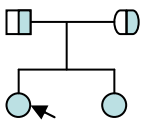
[ ]:number of times mutations identified in affected individuals (note: includes mutations identified in parents of deceased SLO patients.

a: Novel variant

b: This mutation was homozygous in one patient

Green: severe mutations (nonsense/splice mutations) purple: ?milder mutations (missense mutations)

### Case Study 1:

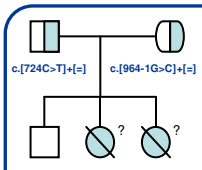



•35 yr old female with short stature, cleft palate, difficulty with feeding, severe learning difficulties, autism and self injury. No biochemistry undertaken. Sister also affected, both diagnosed as children but no follow up. Agreed to accept referral by Psychiatrics after extensive discussion.

•*DHCR7* mutation analysis revealed that this patient is homozygous for an exon 8 missense mutation c.906C>G (p.Phe302Leu), which is one of the 10 most common mutations in Europe [2]. This is proposed to be a transmembrane mutation and therefore the phenotype expected would be at the milder end of the disease spectrum.

•Parental samples not available to establish phase. Diagnosis has alleviated blame of family situation causing self-harming tendencies.

### Case Study 2:



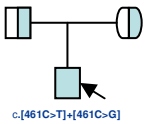

No tissue was available from either foetus therefore *DHCR7* mutation analysis was undertaken on parental samples.

•Unrelated couple, three pregnancies, one normal male and two female foetuses with congenital abnormalities. Foetus 1 – terminated 21 weeks due to scan finding of meningomyelocoele, four limb post-axial polydactyly and growth retardation. Foetus 2 – terminated 19 weeks due to hypoplastic heart, cleft palate, four limb post-axial polydactyly and growth retardation. Both foetus' had the same facial features including short nose, long philtrum and hypertelorism. Referral from Clinical Genetics.

•*DHCR7* mutation analysis revealed the mother to be heterozygous for the common severe exon 9 splice site mutation, c.964-1G>C. The father is heterozygous for an exon 7 missense mutation c.724C>T (p.Arg242Cys) which is proposed to be a transmembrane mutation [5] and is associated with a variable SLOS phenotype [7].

•This couple now have an explanation for the abnormalities identified in their previous pregnancies and prenatal diagnosis can be offered for future pregnancies.

### Case Study 3:




•3 yr old male with moderate developmental delay, talipes, 2/3 toe syndactyly, deep nasal bridge, minimal hypospadias. Abnormal biochemistry: DHC7 levels - 331µmol/L, cholesterol – 3.2mmol/L. Patient referred by Clinical Genetics.

•*DHCR7* mutation analysis revealed that this patient is heterozygous for the exon 6 missense mutation c.451C>T (p.Thr154Met). This mutation has been previously reported and is associated with a reduction of DHCR7 expression below 5% [7]. This patient is also heterozygous for the exon 6 missense mutation c.461C>T (p.Thr154Arg) which has also previously been reported. This patient's phenotype correlates with the predicted phenotype which is expected to be at the mild end of the spectrum from the genotype observed.

## Method & Cost of Analysis

•Bi-directional sequence analysis (ABI3730) of the coding region (exons 3-9) of the *DHCR7* gene. Charge for NHS patients: full gene screen - £362; familial mutation testing £140 (two mutations £185).

## Phenotype/Genotype Correlations

•Phenotype/genotype correlations may exist for this disorder, though other factors can also affect the phenotype such as cholesterol levels [6].
•A milder SLOS phenotype tends to be seen in patients with transmembrane and C-terminus mutations, whereas more severe phenotypes are seen in patients with null mutations and mutations in the 4th cytoplasmic loop [5,7].
•It is believed that homozygosity or compound heterozygosity for the common mutation c.964-1G>C or other null/severe mutations (eg. p.Trp151X) may lead to foetal loss [7], whereas a milder phenotype is more likely associated with missense mutations [5,6].

## Genotype versus phenotype in SLOS: correlations / anomalies observed at BGL

Patient 7 shows that DHC7 levels do not always correlate with the *DHCR7* genotype [8].
Patients 3 and 8 are parents of deceased children who were suspected to have SLOS. BGL has confirmed the diagnosis with *DHCR7* mutation analysis as far as possible where parental samples have been available (patient 8-maternal sample only).
Green: severe mutations (nonsense/splice mutations). Purple: ?milder mutations (missense mutations).
[ ] denotes the four categories of mutations. [0] null mutations; [CT] C terminus mutations (milder); [TM] transmembrane mutations (milder); [L] mutations in the loop domains (L4 is associated with a more severe phenotype) [5].

| Predicted Phenotype | Patient No | Mutation 1 [Mutation category (5)] | Mutation 2 [Mutation category (5)] | Biochemical Diagnosis Y/N (7DHC levels) | Phenotype |
|---|---|---|---|---|---|
| POSITIVE SEVERE | 1 | c.964-1G>A [0] | c.964-1G>A [0] | Y (405µmol/l) | Deceased. Born at 37 weeks. AVSD, short limbs, renal dilatation, ambiguous genitalia, dysmorphic features. |
| | 2 | c.964-1G>A [0] | c.452G>A (p.Trp151X) [0] | Y | Stillbirth at 36 weeks. Previous history of neonatal deaths. |
| | 3 | c.452G>A (p.Trp151X) [0] | N | N | Mother. Deceased child with bilateral renal agenesis. NMD, cleft palate, syndactyly, dysmorphic and sex reversal. |
| | | c.452G>A (p.Trp151X) [0] | N | N | Father – see case above. |
| | 4 | c.964-1G>A [0] | c.1228G>A (p.Gly410Ser) (L8-9) | Y (331µmol/l) | Moderate developmental delay, talipes, 2/3 toe syndactyly, deep nasal bridge, hypospadias. |
| | 5 | c.1210C>T (p.Arg404Cys) (L8-9) | c.546G>A (p.Trp182X) (TM4) novel mutation | Y | Cleft lip/palate, 2/3 syndactyly, microcephaly, micrognathia. |
| | 6 | c.964-1G>A | c.461C>T (p.Thr154Met) | Y (299µmol/l) | 1 yr old male. No clinical information provided. |
| | 7 | c.278C>T (p.Thr93Met) (L1-2) | c.1337G>A (p.Arg446Gln) (CT) | Y (659µmol/l) | 1 year old, typical dysmorphic features, microcephaly, hypotonia, developmental delay, poor feeding. |
| | 8 | c.655T>G (p.Tyr219Asp) (not reported) | N | N | Mother. Daughter deceased – suspected to have SLO. |
| | 9 | N | N | N | Diagnosed with SLO in 1988 – no biochemistry done. |
| MILD NORMAL | 10 | N | N | N | US findings suggest SLO. Phenotypic female with male karyotype. Shortening long bones, chondroplasia punctata. |

## Discussion

•BGL has tested 45 SLOS samples to date including 13 probands where 2 *DHCR7* mutations were identified. Two *DHCR7* mutations have been identified in *all* patients with a biochemical diagnosis of SLOS. 5 patients who are parents of deceased children with suspected SLOS have had a *DHCR7* heterozygous mutation identified (e.g. see case study 2).
•6 BGL patients are pregnant couples where SLOS was suspected in the foetus on scan and mutation analysis was then requested. However, this is not the most efficient testing pathway due to the high cost of *DHCR7* mutation analysis compared to biochemical testing, hence we recommend clinicians to undertake biochemical DHC7 analysis on a prenatal sample if possible prior to genetic testing.
•BGL has reported one novel mutation so far, c.546G>A (p.Trp182X). It is predicted to be disease causing as it creates a stop codon in exon 6, however as it has not been reported previously caution must be used regarding its pathogenicity.
•Our audit has shown that BGL SLOS patient's genotype has correlated with the expected phenotype reported in the literature, though not in every case.
•Large deletions have yet to be found in the *DHCR7* gene, but there are a number of small deletions/insertions that have been described[6] including a 34bp deletion in exon 5 which has been found in a BGL patient.
•Due to the possibility of intrauterine lethality, SLOS is probably underdiagnosed, but as clinicians are becoming more aware of SLOS our referrals are increasing.
**•The information presented demonstrates the importance of this service in aiding the family by providing a definitive diagnosis (important as there are a number of phenocopies), enabling prenatal diagnosis of future pregnancies & to some extent predicting the phenotype.**

## References

1. Porter F (2008) Eur J Hum Genet 16: 535.
2. Witsh-Baumgartner M *et al* (2008) J Med Genet 45:200.
3. Yu H *et al* (2000) Am J Med Genet 90:347.
4. Ryan A *et al* (1998) J Med Genet 35:558.
5. Correa-Cerro & Porter (2005) Mol Genet & Metab 84:112.
6. Yu & Patel (2005)Clin Genet 68:383.
7. Witsh-Baumgartner *et al* (2000) Am J Hum Genet 66:402.
8. Yu *et al* (2000) Hum Mol Genet 9:1385.